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(71)(72) Applicant and Inventor: MANDECKI, Wlodek [PL/US]; 516 Hemlock Lane, Libertyville, IL 60048 (US).

(74) Agent: STEVENSON, Robert, W.; Brinks Hofer Gilson & Lione, NBC Tower, Suite 3600, 455 North Cityfront Plaza Drive, Chicago, IL 60611-5599 (US).

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(54) Title: SCREENING OF DRUGS FROM CHEMICAL COMBINATORIAL LIBRARIES EMPLOYING TRANSPONDERS

(57) Abstract

Materials and methods are disclosed for identifying chemical compounds having desired binding properties towards a binding partner of pharmaceutical interest. The method employs transponders associated with the solid phase material used in the assay and a scanner to encode and decode data stored electronically on the transponder. The data stored on the transponder identifies the monomeric building blocks added during the synthesis. The structural identification of synthesized compounds bound to the solid phase is done by decoding the transponder.

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SCREENING OF DRUGS FROM CHEMICAL COMBINATORIAL LIBRARIES EMPLOYING TRANSPONDERS

BACKGROUND OF THE INVENTION

This invention relates to materials and methods for screening chemical compounds for potential pharmaceutical activity, and more specifically to an electronically-indexed solid phase assay using transponders embedded in the solid phase particles.

Methods of solid-phase chemical synthesis have been widely used in drug discovery. Their advantages include the ease of separating compounds synthesized on the solid phase from the substrates and byproducts of the reactions by centrifugation, filtration, magnetic separation, gravity and others, and the ease of handling and assaying the compounds after the synthesis. Compounds immobilized on the solid phase can be subjected readily to a variety of assays, including immunoassays.

Many classes of compounds have been successfully synthesized using solid-phase methods, including peptides, oligonucleotides and small molecules. There are two general types of compounds that can be synthesized in a combinatorial fashion, linear polymers consisting of one or more monomeric building blocks, e.g. peptides, and compounds built on a small-molecule scaffold. In the latter case the structure of the molecule with regard to the position of the building block can be considered "branched." The latter case is exemplified by compounds from the benzadiazepine family. Hybrid approaches are also possible, where the compounds have a branched structure, but each branch is composed of a linear chain of building blocks, or vice versa.

In existing solid-phase chemical synthesis methods the solid phase is a passive vehicle. The main utility of the solid phase is to aid in separating the desired product or intermediate of the reaction from the substrate and byproducts.

Conventional methods for identification of the synthesized compound are limited because the amount of the compound needed for full characterization (often 1 mg) is much more than the quantity that can be released from a single solid phase particle. One way to overcome this problem is to tag the particle with a biomolecule during the course of the synthesis by co-synthesizing this biomolecule together with the compound of interest. Examples include the co-synthesis of a nucleic acid for peptide combinatorial libraries, the use of halogenated derivatives of carboxylic acids, or the co-synthesis of peptides as tags for small molecules. This method also has limitations, however, in that the chemistry of the synthesis of small molecules may be incompatible with the chemistry of molecular encoding. Additionally, using a molecular tag can double the number of synthetic steps involved in the process.

SUMMARY OF THE INVENTION

This invention overcomes many of these problems by employing transponders embedded in the particles used as the solid phase. Thus, each individual solid phase particle can be assigned a unique index number, electronically encoded inside the particle, that can be recovered at will at any time after the synthesis is complete, thus enabling the identification of the compound on the bead.

According to the present invention, the solid phase performs an additional function, i.e. storage of the information about the progress of the chemical synthesis in an electronic memory element inside the solid-phase particle, or bead. The contents of the memory can be retrieved by a dedicated scanner device at a desired time after the synthesis is completed, or at multiple times during the synthesis, or monitored continuously during an assay. In the combinatorial chemical synthesis methods of this invention, the solid-phase particles are derivatized with a chemical scaffold, or support, on which synthetic condensations are conducted.

Following combinatorial synthesis, the pool of

derivatized particles can be exposed to a target protein of pharmaceutical interest to investigate which particles carry compounds that bind to the target protein, using immunoassay techniques. The particle can also be a source of a small quantity of the compound of interest for identification purposes. The identified compounds can be re-synthesized and subjected to further phases of drug development.

In one aspect, the present invention provides a particle for use in solid phase chemical synthesis, wherein a transponder is embedded in the particle.

In a second aspect, the present invention provides methods of solid phase chemical synthesis employing an electronically indexed solid phase.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a schematic depiction of a solid phase particle having a transponder and a carrying a molecular scaffold for combinatorial synthesis.

FIG. 1A is a schematic depiction of a combinatorial synthesis procedure of this invention.

FIG. 2 is a schematic depiction of a solid phase particle containing a transponder for use in solid phase combinatorial synthesis.

FIG. 3 is a schematic diagram of the signal pathway for encoding and encoding data on the transponders embedded in the solid phase.

FIG. 4 is a schematic representation of a miniature transponder.

FIG. 5 is a plan view of a miniature transponder.

FIG. 6 is a schematic diagram of a procedure for screening synthesized compounds for binding to biomolecular targets of pharmaceutical interest.

FIG. 7 is a plan view of a transport system and analytical instrument for implementing this invention.

FIG. 8 is a plan view of a modified flow cytometer for high speed analysis of solid phase particles of this invention.

DETAILED DESCRIPTION OF THE INVENTION

FIG. 1 depicts an electronically indexed solid phase particle having a transponder for use in combinatorial synthesis on a molecular scaffold. The particle **10** has a transponder **12**, and a surface **16** of the particle **10** is derivatized to display a small molecule scaffold **15** having active groups **13a**, **13b** initially protected with protecting groups **15a**, **15b**. Active groups **13a**, **13b** become the condensation points after deprotection.

Scaffolds for the combinatorial synthesis of small molecules that have been implemented in the art include benzadiazepines, hydantoins, quinolones, alkynes, alkenes, benzoisothiazolones, amides, ureas, tetrahydrofurans, biaryls and others. The scaffold typically contains two to four active groups, that are orthogonally protected, but that can be selectively deprotected and subjected to condensations involving a set of low-molecular weight chemicals, often of similar chemical reactivity.

According to principles of combinatorial chemistry, after the particles are derivatized, the scaffold is deprotected at the first reactive group, and the particles are divided into groups, the number of groups being equal to the number of condensations to be done in parallel. The groups of particles undergo chemical coupling, each group with a different coupling reagent. The particles are then mixed thoroughly, the second reactive group is deprotected, and the particles are again divided into groups to undergo the second coupling step. The procedure is repeated until condensations on all active groups of the scaffold are completed.

In FIG. 1A, a set of transponders **12** is split into groups **12a**, **12b**, the number of groups being equal to the number of different synthetic building blocks **14a**, **14b**. The groups of transponders **12a**, **12b** are electronically encoded with a scanner device (not shown) with an alphanumeric character identifying the first building block to be condensed on the support. The protecting group **15a** is removed and the

groups of transponders **12a, 12b** are subjected to a condensations with the building blocks **14a, 14b**. Often, the condensations are done in parallel. The protecting group **15b** is removed, the second cycle of encoding and synthesis takes place, and the process continues for as long as desired or practical. After the final condensation the scaffold is not cleaved from the support.

Fig. 2 depicts a solid phase particle **10** of the present invention, having a transponder **12** embedded in the particle **10** and a peptide **17** attached to the outer surface **16** of the particle **10**.

A transponder is a radio transmitter-receiver activated for transmission of data by reception of a predetermined signal. The signal comes from a dedicated scanner that also receives and processes the data sent by the transponder in response to the signal. The scanner can also be used to perform the "write" function, i.e., the process of encoding the data on the transponder. Such a combination instrument is referred to as a scanner read/write device. An advantage of the transponder-scanner system is that the two units are not electrically connected by wire, but instead are coupled inductively, i.e. by the use of electromagnetic radiation, typically in the range from 5-1,000 kHz, but also up to 1 GHz and higher.

Figure 3 is a flow chart illustrating the communication between the transponder **12** and a remote scanner read/write device **18**. The transponder **12** embedded in the solid phase beads **10** is encoded with data sent by electromagnetic waves from a remote scanner read/write device **18**. After the assay steps are completed, the beads **10** are analyzed to detect the presence of a label indicative of binding of analyte and those that show the presence of the label are decoded. The scanner **18** sends a signal to the transponder **12**. In response to the signal, the transponder **12** transmits the encoded data to the scanner **18**.

The transponders must be programmable to allow the user to independently encode index numbers in the same number

or a larger number of independent electronic memory fields as the number of condensation cycles. The encoding process at any particular cycle should not change or erase the encoding done in the previous cycles. Accordingly, it is necessary to shield non-target transponders from the electromagnetic radiation emitted by the scanner device, as by a metal barrier, or other means.

Some transponders similar to the Bio Medic Data Systems Inc. (BMDS, 255 West Spring Valley Ave., Maywood, New Jersey) manufactures a programmable transponder for use in laboratory animal identification. The transponder is implanted in the body of an animal, such as a mouse. The transponder is glass-encapsulated to protect the electronics inside the transponder from the environment. One of the transponders manufactured by this corporation, model# IPTT-100, has dimensions of 14 x 2.2 x 2.2 mm and weighs 120 mg. The transponder is user-programmable with up to 16 alphanumeric characters, the 16th letter programmable independently of the other 15 letters, and has a built-in temperature sensor as well. The electronic animal monitoring system (ELAMS) includes also a scanner read/write system, such as the DAS-5001 console system, to encode or read data on/from the transponder. The construction of the transponder and scanner is described in U.S. Patent Nos. 5,250,944, 5,252,962, and 5,262,772, the disclosures of which are incorporated herein by reference. Other similar transponder-scanner systems include the multi-memory electronic identification tag (U.S. Patent 5,257,011) manufactured by AVID Corporation (Norco, CA) and a system made by TEMIC-Telefunken (Eching, Germany). AVID's transponder has dimensions of 1 mm x 1 mm x 11 mm, and can encode 96 bits of information, programmable by the user. The present invention can be practiced with different transponders, which might be of different dimensions and have different electronic memory capacity. The BMDS transponder fulfills the minimum programmability requirements for suitability for combinatorial synthesis.

The commercially available transponders are

relatively large in size. The speed at which the transponders may be decoded is limited by the carrier frequency and the method of transmitting the data. In typical signal transmission schemes, the data are encoded by modulating either the amplitude, frequency or phase of the carrier. Depending on the modulation method chosen, compression schemes, transmission environment, noise and other factors, the rate of the signal transmission is within two orders of magnitude of the carrier frequency. For example, a carrier frequency of 1,000 Hz corresponds to rates of 10 to 100,000 bits per second (bps). At the rate of 10,000 bps the transmission of 100 bits will take 0.01 sec. The carrier frequency can be several orders of magnitude higher than 1,000 Hz, so the transmission rates can be proportionally higher as well.

Therefore, the limiting factor in the screening process is the speed at which the transport mechanism carries the transponders through the read window of the fluorometer/scanner device. The rate of movement of small particles or cells is 10^4-10^5 per second in state-of-the-art flow cytometers. A flow cytometer may be used to practice the present invention, if two conditions are met: (1) the transponders are small enough to pass through the flow chamber, and (2) the design of the flow chamber of the flow cytometer is modified to include an antenna for collecting the electromagnetic radiation emitted by transponders.

A miniature transponder is depicted in Figs. 4 and 5. The source of the electrical power for the transponder **12a** is at least one photovoltaic cell **40** within the transponder **12a**, illuminated by light, preferably from a laser (not shown). The same light also induces the fluorescence of the fluorogenic molecules immobilized on the surface of the transponder **12a**. The transponder **12a** includes a memory element **42** that may be of the EEPROM type. The contents of the memory is converted from the digital form to the analog form by a Digital-to-Analog converter **44** mounted on the transponder **12a**. The signal is amplified by an amplifier **46**,

mixed with the carrier signal produced by an oscillator **48**, and emitted to the outside of the transponder **12a** by an antenna **50**.

The contents of the transponder memory can be permanently encoded during the manufacturing process of the transponder, different batches of transponders being differently encoded. Preferably, the memory of the transponder is user-programmable, and is encoded by the user just before, during, or just after the biological material is deposited on the surface of the transponder. A user-programmable transponder **12a** must have the "write" feature enabled by the antenna **50**, amplifier **44** and the Analog-to-Digital converter **46** manufactured on the transponder **12a**, as well as the dedicated scanner read/write device.

The advantages of this transponder are several-fold. First, the dimension of the transponder is reduced, since most of the volume of current transponders is occupied by the solenoid. The design discussed above will enable the production of cubic transponders on the order of 0.01 to 1.0 mm as measured along a side of the cube, preferably 0.05 to 0.2 mm.

Second, a large number of transponders can be manufactured on a single silicon wafer, and no further assembly would be required to attach the solenoid to the VLSI chip. As depicted schematically in Fig. 6, a silicon wafer **60** is simply cut to yield active transponders **12a**. Third, the transponder, according the new design, will not need the glass capsule as an enclosure, further reducing the size of the transponder. Silicone dioxide (SiO_2) would constitute a significant portion of the surface of the transponder, and SiO_2 has chemical properties which are very similar to glass in terms of the feasibility of derivatization or immobilization of biomolecules. Fourth, and perhaps most importantly, the narrow focus of the beam of the laser light would enable only one transponder to be active at a time, significantly reducing the noise level. Advanced user-programmability is desirable as well, various memory registers need to be addressable

independently (writing in one register should not erase the contents of other registers).

A second preferred embodiment of the present invention comprises a method for the combinatorial synthesis of branched compounds on a scaffold linked to an electronically indexed solid phase. The starting material is a scaffold molecule with several active groups, initially protected with orthogonal protecting groups, immobilized on the transponders through a molecular linker. The first active group is deprotected, and the transponders are divided into groups at random, the number of groups being equal to the number of building blocks. The transponders are electronically encoded with an alphanumeric character unequivocally identifying the first building block to be condensed on the support. Because the building block added to the scaffold will be different for each group, the encoded character is also different for each group of transponders. The groups of transponders are then subjected to condensations with the building blocks, often in parallel. The groups of transponders are combined and mixed thoroughly, the second active group on the scaffold now carrying the first building block is now deprotected, and the transponders are again divided into group at random. The second cycle of encoding and synthesis is performed, and the process is repeated as many times as desired or practical, but only until all active groups on the scaffold have undergone coupling. After the final condensation the scaffold remains on the transponders.

Fig. 6 depicts the analysis of the synthesized compounds bound to transponders. The particles **10** carrying synthesized compounds **19** are subsequently incubated with a labelled biomolecular target **21** of pharmaceutical interest, e.g., a receptor molecule, in a single vessel (not shown) in a standard immunoassay. The label may be fluorescent, colorimetric, radioactivity or the like. After analyzing the transponders to detect the label, the transponders are decoded to determine the structure of the synthesized compound attached to the transponders.

The chemical libraries synthesized on transponders can be subjected to screening for binding to several types of biomolecular targets of pharmaceutical interest, such as proteins and peptides, and their covalently modified forms, protein or peptide conjugates, small molecules (haptens), ribonucleic acid (RNA), modified nucleic acids and analogs of nucleic acids (in particular protein-nucleic acids, PNAs). The target can be a complex of biomolecules, such as a virus particle, a protein-nucleic acid complex, or a protein-hapten complex. The target can be a cell, in such case the relevant molecules that participate in binding process during the assay are typically cell surface receptors or other elements of the cell wall or membrane. The target may be present in a variety of forms, such as a solution in a simple buffer, but also in a complex biological fluid, such as blood, serum, urine, saliva, and many others.

After incubation with the target, the transponders are analyzed to detect the label, and the electronic memory of the transponder is decoded using a dedicated scanner. The two measurements can be done at the same time manually. Alternatively, a single automated instrument can perform both functions. Such an instrument can be a modified fluorometer in which the scanner is mounted in the proximity of the readout window, and the reading of fluorescence and the decoding of the memory of the transponder are coordinated by a central computer.

Figure 7 shows the analytical instrumentation and transport system used in an embodiment of the present invention. A quartz tube **20** is mounted in the readout window **22** of a fluorometer **24**. The quartz tube **20** is connected to a metal funnel **26**. The length of the quartz tube **20** is similar to the dimensions of the transponder **12**. Transponders **12** are fed into the metal funnel **26**, and pass from the funnel **26** into the quartz tube **20**, where the fluorescence is read by the fluorometer **24** and the transponder **12** is decoded by the scanner **27**, and then exit through a metal tube **28** and are conducted to a collection vessel (not shown). The metal

funnel **26** and metal tube **28** are made of metal shield transponders **12** outside of the read window **22** by shielding from the electromagnetic signal from the scanner **27**. This shielding prevents the scanner signal from reaching more than one transponder **12**, causing multiple transponders **12** to be decoded.

Minimal modification of the fluorometer **24** would be needed in the vicinity of the location that the tube occupies at the readout moment to allow for positioning of the transponder reading device. To assure compatibility with existing assays, the glass surrounding the transponder could be coated with plastic currently used to manufacture beads.

In a preferred design, depicted in Fig. 8, a metal coil antenna **30** is wrapped around the flow cell **32** of a flow cytometer **29**. The transponders **12** pass through the flow cell **32**, and are decoded by the scanner device **27**. The signal carrying the data sent from the transponders **12** is amplified by a first amplifier **34** and processed by the scanning device **27**. As the transponders **12** are decoded, fluorescence from the transponders **12** is detected and analyzed by the flow cytometer **29**.

EXAMPLE 1

Synthesis Of Combinatorial Library
Of Peptides On Transponders

A set of 200 transponders, model IPTT-100, manufactured by BMDS is used in this example. The outside glass surface of the transponders is derivatized by treatment with aminoalkylsilane as follows. First, the transponders are cleaned by washing with xylene, followed by a 70% ethanol rinse and air drying. Then, the transponders are submerged for about 30 seconds in a 2% solution of aminopropyltriethoxysilane (Cat.# A3648, Sigma, St. Louis, MO) in dry acetone. The transponders are then sequentially are rinsed with dry acetone and distilled water, and air dried. This procedure is described in the Pierce catalog (pp. T314-T315 of the 1994 catalog, Pierce, Rockford, IL). The aminoalkylsilane treatment results in covalent linkage of the primary amine group to the surface of the transponder.

Peptide synthesis is carried out on the solid support created by aminoalkylsilane treatment, using an Applied Biosystems peptide synthesizer model 431A at a 1 mM scale using standard Fmoc chemistry. The 200 derivatized transponders are divided into 5 groups of 40 transponders, and are inserted into 55 ml reaction vessels. The volume of transponders in each group is about 2 ml.

First, a six-residue peptide linker, common to all five groups, is synthesized, corresponding to the following sequence: NH_2-Met-Phe-Gly-Cys-Ser-Gly. Second, each group undergoes a single coupling reaction, to join one residue to the growing polymer. Specifically, the following residues are added:

Group 1: Ala
Group 2: Arg
Group 3: Asp
Group 4: Gly
Group 5: Ser

Following these condensations, the transponders are removed from the reaction vessels and each transponder of each group is assigned an index number indicating the residue added in

the first condensation reaction. The transponders are then pooled in a common vessel, mixed thoroughly, and again divided into five groups of forty transponders. The groups again undergo a single condensation, as above, and the transponders are again encoded with a second index number identifying the residue added in the second coupling step. Finally, five coupling reactions, common to all 200 transponders, are carried out to add the following sequence: NH_2-Ser-Gly-Ser-Cys-Arg.

The results of the synthesis should provide all 25 possible combinations of Ala, Arg, Asp, Gly and Ser at the 7 and 8 positions, as shown below:

Ala Ala
Arg Arg
NH_2-Ser-Gly-Ser-Cys-Arg-Asp-Asp-Met-Phe-Gly-Cys-Ser-T
Gly Gly
Ser Ser

where T represents the transponder surface. The transponders are then removed from the reaction vessels, pooled and deprotected using standard methods. The peptides are not cleaved from the transponders because no labile bond is present. The peptides are oxidized by incubating the transponders in 10 mM ammonium acetate pH 8.5 overnight at room temperature with gentle stirring, are then rinsed and stored in phosphate-buffered saline.

The synthesized peptide library is screened against platelet glycoprotein IIb/III, a mediator of aggregation of platelets through binding of fibrinogen. The IIb/III protein is radio-labelled with ^{125}I. The 200 transponders carrying the peptide library are submerged in a solution of ^{125}I-labelled IIb/III protein in phosphate-buffered saline (PBS), and incubated at room temperature for 2 hours. The volume of the solution is sufficient to immerse the transponders. The transponders are rinsed five times with PBS. The transponders are then analyzed to detect the label radioactivity, and the transponders are decoded to yield the structure of the synthesized peptide bound thereto.

I claim:

1. A method of solid phase chemical synthesis employing solid phase particles having transponders, the transponders having memory elements, wherein an index number indicating the structure of the synthesized compound is encoded on the transponder memory element.

2. The method of claim 1, further comprising screening the synthesized compounds for binding to a target biomolecule of pharmaceutical interest.

3. The method of claim 1 wherein the synthesized compound is a peptide.

4. A method of solid phase chemical synthesis, comprising the steps of:

(a) providing a solid phase particle having a transponder, and having a chemical scaffold attached to a surface of the particle, the scaffold having active groups; and

(b) sequentially adding chemical substituents to the active groups on the scaffold, and encoding an index number on the transponder to indicate each substituent added.

5. The method of claim 4, further comprising the steps of:

(a) incubating the particle having a synthesized compound attached thereto with a labelled target biomolecule of pharmaceutical interest;

(b) analyzing the particle to detect the labelled target molecule; and

(b) decoding the transponder to determine the structure of the synthesized compound.

6. A method of screening for pharmaceutically-active compounds, comprising the steps of:

(a) providing a particulate solid phase having transponders associated with the particles and a chemical scaffold attached to a surface of the particles, the transponders having memory elements, the scaffold having active groups;

(b) dividing the particles randomly into groups;

(c) sequentially adding chemical substituents to the active groups on the scaffold, encoding an index number on the transponder for each addition to indicate the substituent added, combining the groups and re-dividing the particles into groups after each addition;

(d) repeating the addition, combining and re-dividing steps until all active groups on the scaffold have been reacted;

(e) incubating the particles having a synthesized compound attached thereto with a labelled target biomolecule of pharmaceutical interest;

(f) analyzing the transponder to detect the labelled target molecule; and

(g) decoding the transponder to determine the structure of the synthesized compound.

Fig. 1

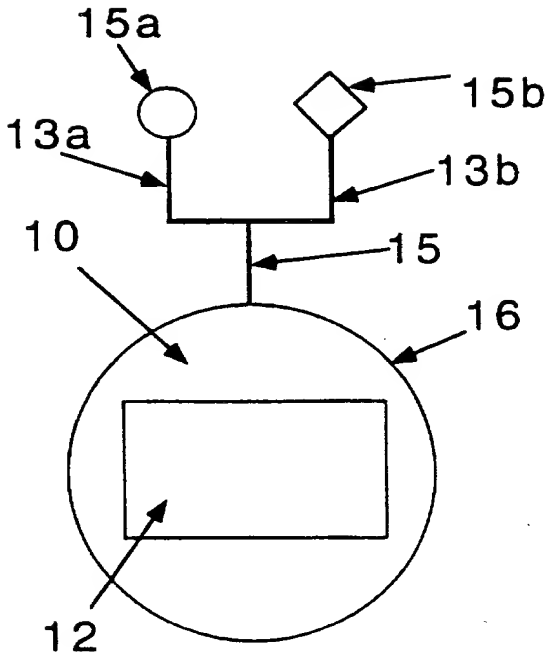
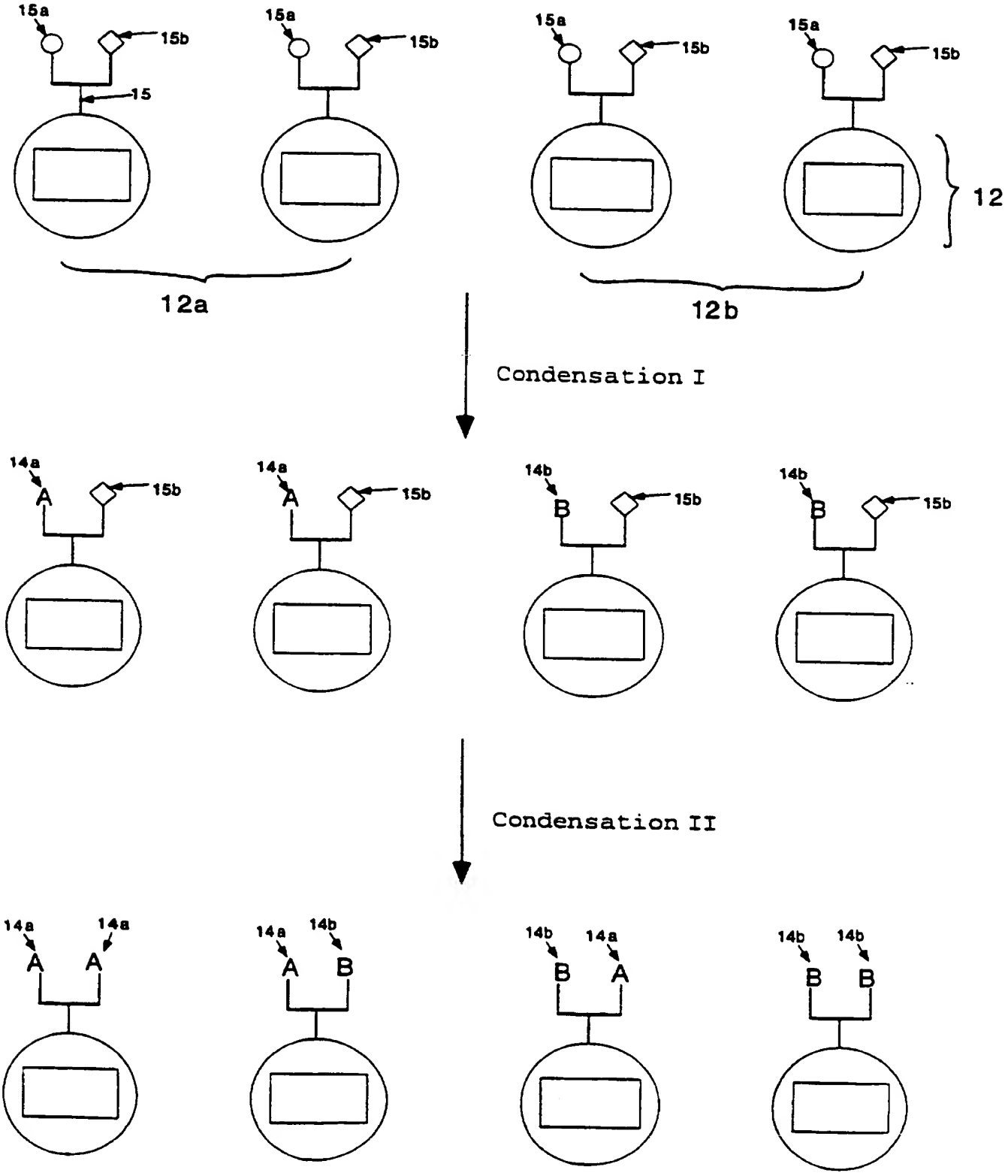


Fig. 1A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15b
15
12a
12b
12
Condensation I
14a
14b
A
B
Condensation II

Fig. 2

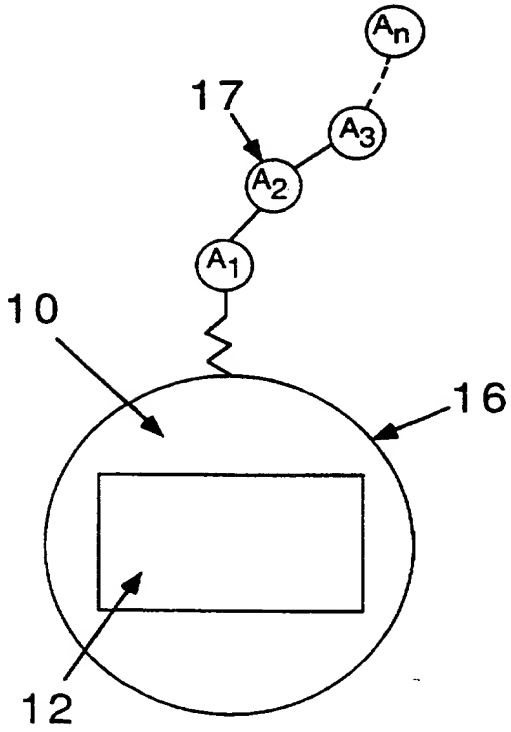


Fig. 3

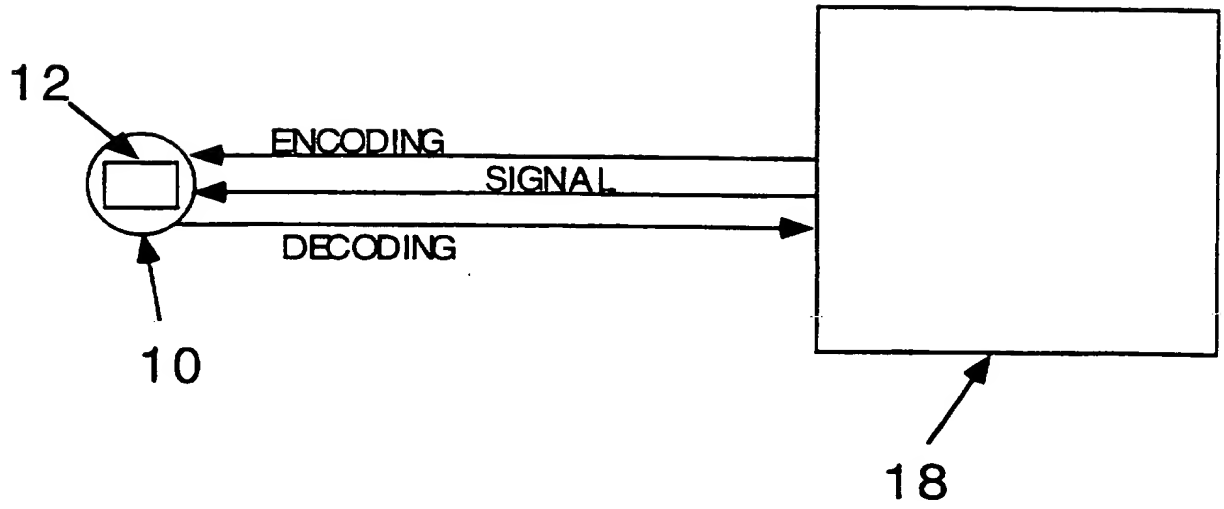


Fig. 4

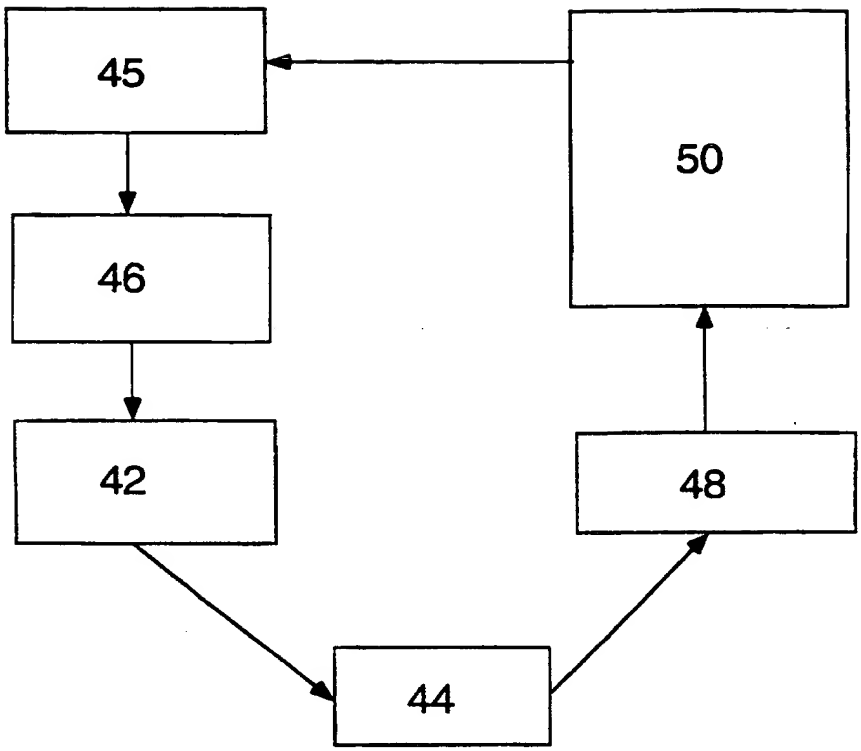


Fig. 5

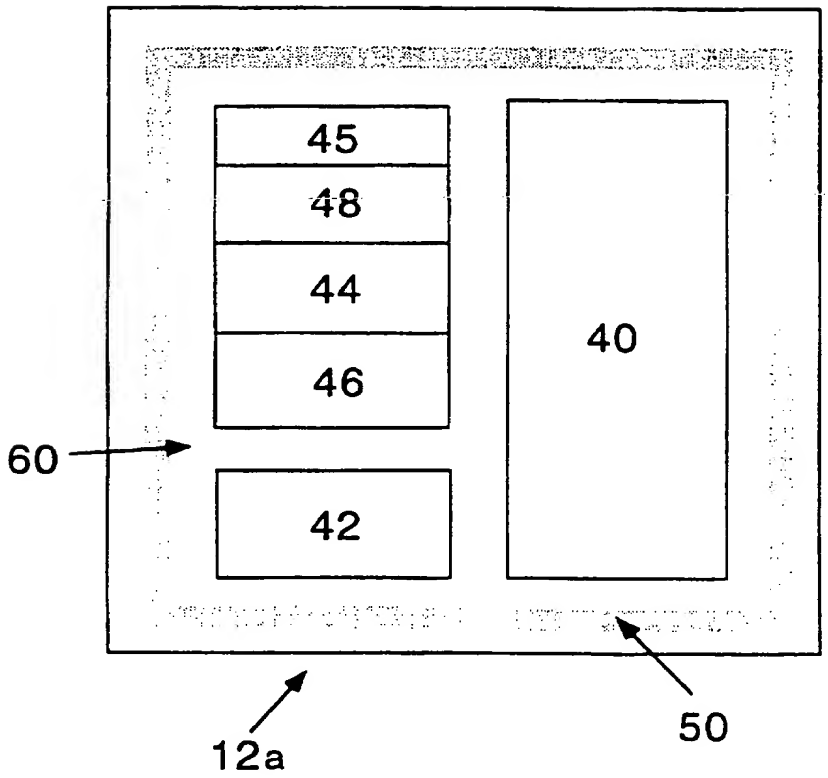


Fig. 6

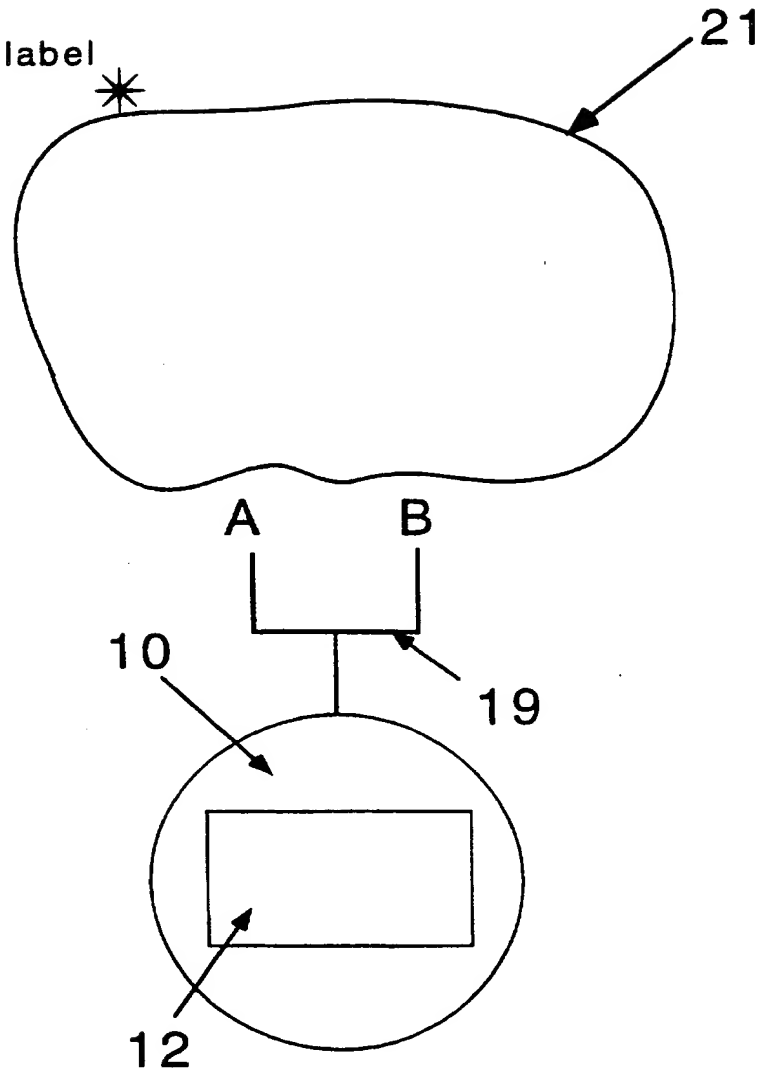


Fig. 7

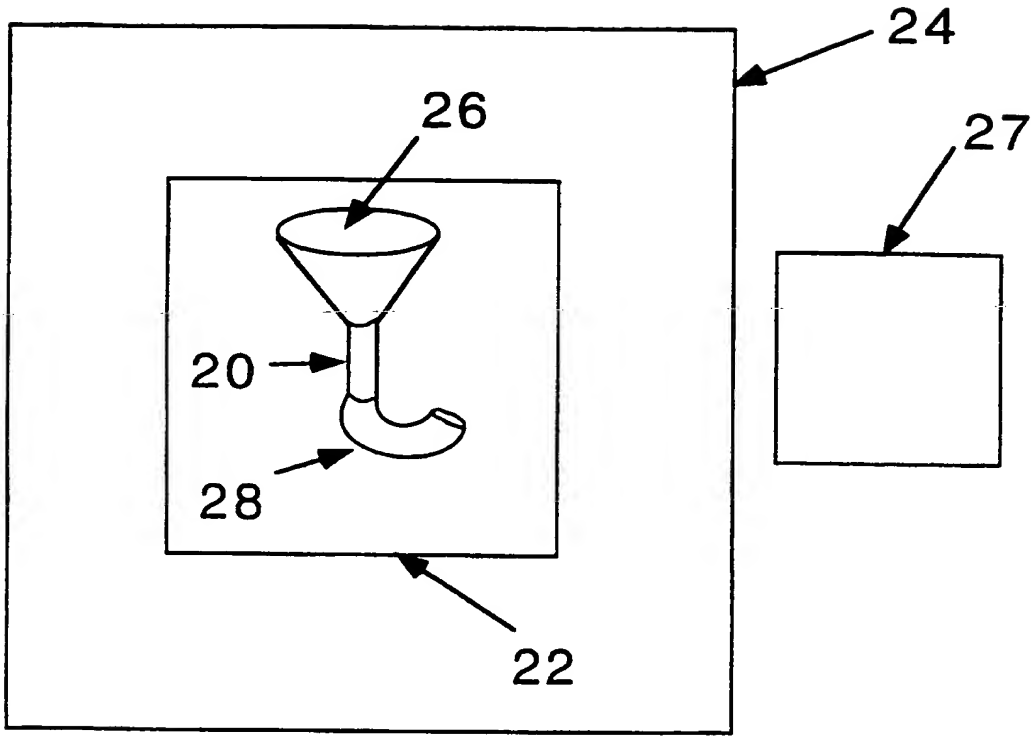
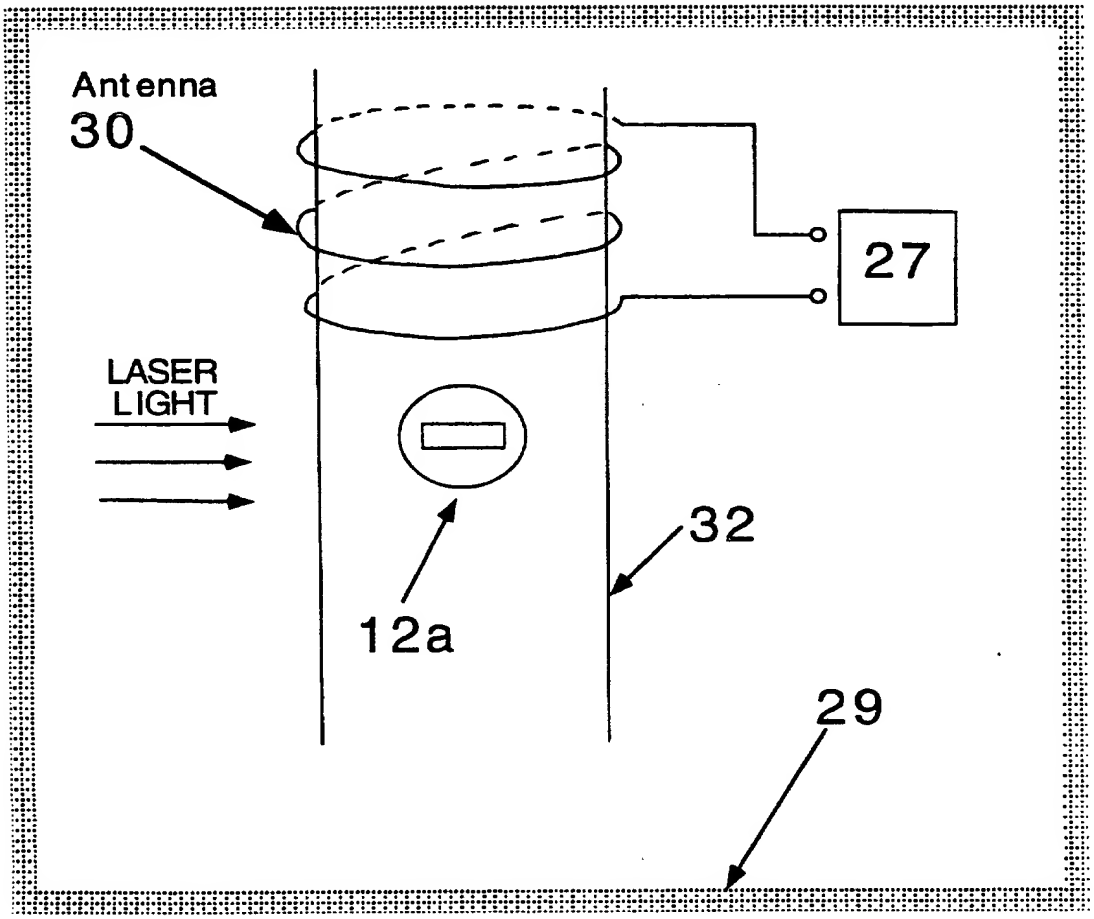


Fig. 8



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18940

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 17/00; A61K 38/00
US CL :530/334
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/333, 334; 536/25.3; 427/2.11; 435/7.1; 436/501, 518, 523, 531, 534

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG
Search terms: transponder? ? and (synthesis or synthesiz?); microchip? ? and (synthesis or synthesiz? ?)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Science, Volume 270, issued 27 October 1995, Robert F. Service, "Radio Tags Speed Compound Synthesis," page 577, especially column 1, first paragraph and column 3, first and second paragraphs.	1-6
A	Science, Volume 264, issued 03 June 1994, Joseph Alper, "Drug Discovery on the Assembly Line," pages 1399-1401, especially page 1399, column 2, first paragraph and column 3, second full paragraph, and page 1400, column 1.	1-6

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of th international search	Date f mailing of the international search report
26 FEBRUARY 1997	18 MAR 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile N . (703) 305-3230	Authorized officer SUSAN C. WOLSKI Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/18940

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, f the relevant passages	Relevant to claim No.
X ---- Y	Journal of the American Chemical Society, Volume 117, issued 1995, Edmund J. Moran, "Radio Frequency Tag Encoded Combinatorial Library Method for the Discovery of Tripeptide-Substituted Cinnamic Acid Inhibitors of the Protein Tyrosine Phosphatase PTP1B," pages 10787-10788, especially page 10787, column 2, and page 10788, column 1.	1-4 ----- 5-6
A	Nature, Volume 354, issued 07 November 1991, Kit S. Lam et al., "A new type of synthetic peptide library for identifying ligand-binding activity," pages 82-84, especially page 82, column 2, first paragraph.	1-6